

# The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal

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**Abstract.** Plants contain a variety of chemical defenses that strongly affect feeding rates in captive mammals, but their effects on the fitness of wild herbivores are largely unknown. This is because the complexity of defensive compounds, and herbivores' counteradaptations to them, make their effects in the wild difficult to measure. We show how tannins interact with protein to produce spatial variation in the nutritional quality of eucalypt foliage, which is related to demography in a wild population of a marsupial folivore, the common brushtail possum (*Trichosurus vulpecula* Kerr). Tannins reduced the digestibility of nitrogen (N) in vitro, creating variation in available N concentrations among the home ranges of individual possums in an otherwise homogeneous habitat. This was strongly correlated with reproductive success: females with better quality trees in their home range reproduced more often and had faster-growing offspring. These results demonstrate a powerful mechanism by which spatial variation in plant chemistry may control herbivore population dynamics in nature.

**Key words:** common brushtail possum; Eucalyptus spp.; herbivory; marsupial; nutrition; plant defense; population dynamics; primates; protein; reproductive success; tannins; *Trichosurus vulpecula*.

## INTRODUCTION

The nutritional value of browse to herbivores is often compromised by the presence of plant secondary metabolites (PSMs). These compounds may act as toxins or feeding deterrents that reduce the palatability of plants, or as digestibility-reducers that decrease the availability of nutrients (Iason 2005). Experiments with captive animals have shown that PSMs limit feeding in a range of mammal species (e.g., Villalba et al. 2002, Moore et al. 2005, Sorensen et al. 2005); however, their effects have never previously been linked to variation in fitness and demography in wild populations of mammalian herbivores. This is because the complexity of defensive compounds, and herbivores' counteradaptations to them, make their effects in the wild difficult to measure. Also, natural concentrations of these compounds are normally distributed and spatially correlated within plant species (Lawler et al. 2000, Brenes-Aguedas and Coley 2005, Andrew et al. 2007), so animals rarely encounter the extreme concentrations that characterize captive studies.

Studies in forests worldwide have suggested that links between soil fertility and plant defense can result in patchy distributions of animals across landscapes (McKey et al. 1978, Oates et al. 1990, Cork 1992,

Ganzhorn 1992, Bryant 2003). Thus, it is often assumed that PSMs have fitness consequences for herbivorous mammals, an assumption that is fundamental to ideas about evolutionary "arms races" between plants and herbivores. However, evidence for this is lacking. Demonstrating effects of plant defensive chemicals on the fitness of individual browsers has important implications for understanding herbivore population dynamics and plant-herbivore coevolution. We carried out a study over five breeding seasons to investigate how defensive chemistry in eucalypts impacted reproductive success in a wild population of a marsupial folivore, the common brushtail possum (*Trichosurus vulpecula* Kerr).

Common brushtail possums are small (1.5–4.5 kg), generalist herbivores that have the widest distribution of any of the arboreal marsupials. They inhabit a broad range of habitat types, from tropical savannas, to the wet sclerophyll forests of southeastern Australia, as well as urban areas (Kerle 2001). Brushtail possums are a solitary, nocturnally active species and their biology and life history has been well documented, particularly with respect to populations in northern Australia (e.g., Isaac and Johnson 2003, Isaac 2005). Most populations exhibit a breeding peak in the autumn, with females producing a single young that remains in her pouch for about five months. A second, smaller breeding peak may occur in the spring in some populations, but not all females breed twice per year (Kerle 2001). Common brushtail possums incorporate varying amounts of *Eucalyptus* into their diet. In the northern savannas, eucalypt foliage is the primary dietary component,

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whereas in other habitats, possums consume a mixed diet consisting of leaves from many plants, fruits, flowers, and grasses (Kerle 2001).

Diet selection in captive brushtail possums is strongly influenced by concentrations of a class of phenolic PSMs found in many eucalypts, the formylated phloroglucinol compounds, or FPCs (Wallis et al. 2002), but they are also very sensitive to tannins (Marsh et al. 2003). Eucalypts also contain terpenoids, but captive studies have shown that they impose few detoxification costs to brushtail possums and do not primarily influence diet selection (McLean and Foley 1997, Lawler et al. 1999). Tannins can bind to proteins, thereby reducing the availability of N to herbivores (Robbins et al. 1987). Tannins are most likely to affect the nutritional status of animals foraging in the wild, because they are ubiquitous in eucalypts (Fox and Macauley 1977). In contrast, FPCs primarily act as feeding deterrents, influencing tree choice by marsupial folivores (Scrivener et al. 2004, Moore and Foley 2005). We lack studies that recognize the impact of PSMs on nutrient availability, even though this is the mechanism by which they are most likely to influence herbivore fitness.

Ecologists often attempt to relate herbivore performance to concentrations of leaf N, which is used as a proxy for protein content (White 1993). However, the negative effect of tannins on N availability, via protein-binding, means that this approach greatly overestimates the availability of N to animals. Previously, we developed a new in vitro method that integrates measures of the negative effects of tannins and plant fiber to estimate the amount of N that is available to possums, i.e., digestible N (DeGabriel et al. 2008). In that study, we showed that concentrations of digestible N in natural eucalypt woodlands in northern Australia were significantly lower than total N concentrations, and that the two measures were not correlated at a landscape scale. However, reductions in N digestibility were correlated with tannin concentration (DeGabriel et al. 2008). Studies with captive animals have demonstrated that tannins significantly reduced the digestion of N in *Eucalyptus melliodora* leaves by brushtail possums by 25.9–40.5% (Marsh et al. 2003), so we would expect tannins to affect their performance in the wild. Similarly, the apparent N digestibility of *E. punctata* (D.C.) foliage by a eucalypt specialist, the koala, was only 45% (Cork et al. 1983), so measuring total N is not accurately indicative of the nutritional benefit of foliage to animals.

To test how variation in the quality of trees available to individual female possums affected their reproductive success, we applied our in vitro measure of digestible N together with three other measures of foliar chemistry, described in DeGabriel et al. (2008): polyethylene glycol binding capacity (PEG-BC, which provides an indication of tannin activity), in vitro dry matter (DM) digestibility, and total leaf N. We used near-infrared reflectance spectroscopy (NIRS) to predict values of these traits in a large number of trees within each

possum's home range, enabling us to more accurately describe the foraging environment encountered by wild possums (Foley et al. 1998). We then investigated whether variation in home range quality between females influenced the number of offspring that they produced and the growth rates of their offspring.

## MATERIALS AND METHODS

### *Study site and vegetation composition*

We conducted this study between October 2004 and December 2006 at Tabletop Station, near Townsville in the dry tropics of north Queensland (19°23' S, 146°27' E; 400 m above sea level). The vegetation structure at the site was relatively simple, consisting of open eucalypt (species of the genera *Eucalyptus* and *Corymbia*) woodland with a grassy understory. The dominant tree species was the narrow-leaved red ironbark (*Eucalyptus drepanophylla* (F. Muell.)), with *Corymbia tessellaris* (F. Muell.), *E. platyphylla* (F. Muell.), *E. tereticornis* (Smith), *C. dallachyana* (Benth.), *C. erythrophloia* (Blakely), and *C. intermedia* (R. T. Baker) also common. A mixture of other plant species including *Melaleuca* spp., *Lophostemon* spp., *Ficus rubiginosa*, and *Euroschinus falcata* were also present, but at relatively low abundance.

To describe the vegetation composition, we performed four band transects, each 500 m long and 4 m wide. Along the length of each of these, we recorded the diameter at breast height (130 cm) and species of all woody plants and standing dead trees greater than 5 cm dbh if their center point fell within the bounds of the band.

### *Trapping protocol and determination of female reproductive success*

Common brushtail possums can breed a maximum of twice per year, (Kerle 2001), so our study spanned five potential breeding events. We established a trapping grid over ~50 ha, with traps placed 50 m apart. We trapped possums over 4–5 nights at approximately 6-week intervals using wire cage traps, baited with a mixture of peanut butter, oats, and flavoring essence. Upon first capture, we permanently tagged all possums with a subcutaneous passive integrated transponder (PIT) tag (Trovan, Melbourne, Australia) and estimated their age using the tooth-wear index developed for *T. vulpecula* by Winter (1980). During every trapping session, we measured the head length of each possum to the nearest 0.1 mm and recorded their body mass to the nearest 25 g.

In each trapping session we assessed the reproductive status of females and recorded the sex and stage of development of un-weaned juveniles. We measured the head lengths of pouch young (PY) to the nearest 0.1 mm and assigned them dates of birth using growth equations developed by Isaac and Johnson (2003) for north Queensland possum populations. As PY head growth was linear until pouch emergence, we calculated growth rates for PY that we had measured at least twice while in the pouch, by dividing the change in head length by the time interval between each measurement and averaging

across all measurements. We scored the breeding success of each female as the percentage of observed breeding seasons that she successfully reared a PY to pouch emergence. Offspring known to have died before pouch emergence were not counted as a successful breeding attempt. We developed an index of body condition for populations of female brushtail possums in north Queensland using the residuals from a semiparametric local polynomial regression (LOESS regression) model of body mass on head length with the software package R (base package; R Core Development Team 2004). We used this index to determine the body condition of females at every capture and then averaged all predictions for each female over the duration of the study.

#### *Home range analysis*

We fitted collar-mounted single-stage radio transmitters (Sirtrack Wildlife Tracking Solutions, Havelock North, New Zealand; 6 g mass) to all adult female possums caught between November 2004 and August 2005 ( $n = 20$ ; mean body mass = 1820 g). We radio-tracked possums over 51 nights, divided across three sessions (January–April 2005, October 2005, and February–April 2006), to determine the trees used by individual possums and to calculate their home ranges. We located each collared possum at least once per night, spacing our fixes over many hours and tracking the possums in a different order each night to minimize bias. We pooled the fixes from each session in order to amass data of possum habitat use over at least one year for each individual, collecting between 8 and 49 fixes per female (mean = 31; total = 594). Upon locating each possum, we assigned to the tree a unique number, recorded the tree species, its diameter at 130 cm, and whether the animal was feeding. We recorded the locations of radio-tracking fixes to within 5 m using a hand-held differential GPS (Garmin, GPS 72) and averaged multiple GPS coordinates taken at each point. We then mapped the fixes using OziExplorer GPS mapping software (Brisbane, Australia) and ArcView 3.3 (ESRI, Redlands, California, USA). We used minimum convex polygons to determine the 90% home range of all females with  $\geq 20$  fixes after discarding the 10% of observations farthest from the harmonic mean of locations for each individual, using the Animal Movement Extension (Hooge and Eichenlaub 1997) in ArcView 3.3 ( $n = 16$  female possums). The aim of these analyses was to determine the core area used for foraging by each possum, to allow us to partition the trees that were potentially available for it to use. This approach allowed us to obtain a more representative description of the foraging environment of individual females than focusing only on trees that we had observed possums using.

#### *Analyses of leaf chemistry*

We collected fresh leaves from all eucalypts used by possums and the nearest paired tree of the same species

to each. We freeze-dried and ground all samples and then analyzed PEG-BC, total leaf N concentration, and in vitro DM and N digestibility for a subset (see the Appendix for sample sizes), following DeGabriel et al. (2008), to enable us to develop NIRS calibrations. Briefly, we used radio-labeled PEG to measure the affinity of foliage samples for PEG (PEG-binding capacity), which provides a measure of tannin activity (DeGabriel et al. 2008). We then carried out a two-stage in vitro digestion of foliage using pepsin and cellulase to mimic digestion in the animal's gut. *E. drepanophylla* samples were digested in centrifuge tubes, in both the presence and absence of PEG, to allow us to quantify the effects of tannins on N digestibility. The difference in digestibility of samples digested with and without PEG was determined to be the effect of tannins. Samples of species from the eucalypt genus *Corymbia* were digested in bulk in ANKOM filter bags without the addition of PEG, using the simplified method described in DeGabriel et al. (2008). We determined the N concentration of both the original leaf sample and the digested residue using the Kjeldahl method and subtracted the value for the residue from the N concentration of the leaf to calculate what percentage of N was digested (N digestibility). We calculated digestible N concentration (%) by multiplying the N digestibility of leaf samples by their total N concentration. We calculated DM digestibility by subtracting the mass of the digested residue from the initial sample mass and dividing this by the initial mass (DeGabriel et al. 2008). Analysis of FPCs followed the HPLC method described by Wallis and Foley (2005).

We recorded the reflectance spectrum of each sample between 400 and 2498 nm using an NIRSystems 6500 scanning spectrophotometer with spinning cup attachment (Foss, Silver Spring, Maryland, USA), according to Moore et al. (2004). We developed NIRS calibrations to predict leaf N concentration, PEG-BC, N digestibility, digestible N concentration, and DM digestibility using the software WinISI 3, version 1.50E (Infrasoft International, Port Matilda, Pennsylvania, USA), as described in DeGabriel et al. (2008). Summary statistics for the final NIRS models are given in the Appendix. We determined the average values for each of the nutritional variables in all trees sampled within each female's 90% range, including both used and unused trees of all eucalypt species. We predicted concentrations of these traits in 598 trees, representing 23–68 trees (mean = 40) across each female's home range. We only considered possums for which we had data for more than 20 trees ( $n = 15$ ). Table 1 provides details of the home ranges of each of the female possums included in the study.

#### *Statistical analyses*

We used one-way ANOVA to test for differences between the values of PEG-BC, digestible N, total N, and DM digestibility in the trees sampled in each female's home range. We then used ordinary least

TABLE 1. Summary of the home ranges of the female possums (*Trichosurus vulpecula*) included in the study.

Possum	Home range size (ha)	No. radio-tracking locations	No. trees sampled	Mean digestible N concentration (% DM)	Mean total N concentration (% DM)	Mean PEG-BC (g/100 g DM)	Mean DMD (%)
834F	0.83	20	23	0.390	1.021	13.723	51.511
12O4	0.51	21	25	0.372	1.203	11.318	40.232
68D1	1.96	35	29	0.420	1.033	12.406	48.033
E91A	2.32	31	31	0.413	1.135	12.034	46.375
80FC	3.49	37	35	0.348	1.058	11.928	43.887
2092	1.43	37	32	0.402	1.120	12.165	45.723
9A50	1.99	47	33	0.293	1.274	11.816	35.833
A177	0.81	37	39	0.364	1.140	11.074	41.806
D189	2.95	43	39	0.418	1.079	12.077	45.772
34F6	1.66	37	41	0.479	1.081	11.339	48.159
5B0E	3.67	34	42	0.327	1.096	12.120	41.115
7923	4.70	26	43	0.374	1.073	12.835	48.889
DD34	2.81	48	67	0.395	1.053	12.447	47.517
AA9A	2.28	45	68	0.439	1.079	12.225	48.618
7159	2.66	26	45	0.385	1.033	12.90	50.420

Note: Abbreviations are: PEG-BC, polyethylene glycol-binding capacity; DM, dry matter; DMD, dry matter digestibility.

squares (OLS) regression to test the effects of PEG-BC and total leaf N on digestible N concentrations in the dominant tree species, *E. drepanophylla*. We also used OLS regression to test the relationship between home range size and home range quality.

We used univariate and multivariate analyses to test the effects of the four nutritional variables, body condition, and maternal age and size (head length) on breeding success. Because our data were binomial, we used logistic regression models, incorporating a logit-link function, with breeding success as the response variate. Test statistics were considered significant at  $P \leq 0.05$ . For multivariate analyses, we used Akaike's information criterion, AIC (Akaike 1973), to select the model that best described variation in breeding success.

In order to test the effects of nutritional variables and maternal age, body condition, and size on offspring growth rates, we used linear mixed models with PY head growth rate as the dependent variable. Because we had measured multiple offspring for each female, and thus our data points were not independent, we included mother's identity as a random term in the models, because we had insufficient data to perform repeated-measures analyses. We used one-way ANOVA to determine the effects of offspring sex on growth rates of 14 male and 13 female PY. All analyses were performed in GenStat, ninth edition (VSN International 2006).

## RESULTS

### *Vegetation structure and variation in home range quality*

Our vegetation transects confirmed that *E. drepanophylla* was the dominant tree species at the site, accounting for 42.9% of the basal area measured, followed by *E. tereticornis* (24.9%), *Corymbia* spp. (11.8%), *E. platyphylla* (10.9%), and unidentified eucalypts and other plant species (9.5%). However, reporting the vegetation structure in this way may greatly overestimate the importance of *E. tereticornis*, as we

only recorded the presence of a single, very large tree on our transects. Counting the number of stems of each species per hectare indicated that *E. drepanophylla* was the dominant species (55.2% of all trees), followed by *Corymbia* spp. (27.6%), non-eucalypts and unidentified species (13.6%), *E. platyphylla* (2.8%), and *E. tereticornis* (0.8%).

Tree use by possums reflected species abundance at the site. We observed female possums most frequently in *E. drepanophylla* (61.5% of observations), followed by *Corymbia* spp. (14.7%), non-eucalypts (11.4%), *E. platyphylla* (7.8%), and *E. tereticornis* (4.6%). Of our 583 arboreal records of possums, we observed them feeding on 48% of occasions. Similar to our observations of tree use, we found that *E. drepanophylla* was the most important dietary component (63.6%), followed by *Corymbia* spp. (16.1%), *E. platyphylla* (8.2%), non-eucalypts (6.4%), and *E. tereticornis* (5.7%).

Because 76.1% of all arboreal observations of possums and 86% of observations of possums in eucalypts were in *E. drepanophylla* and the four *Corymbia* species, we restricted our analyses to these species. These species contained no FPCs, but varied in their PEG-BC. In addition, the two most common tree species, *E. drepanophylla* and *C. tessellaris*, contain only trace amounts of terpenoids (Bignell et al. 1997a, b), so we were able to attribute any negative effects to the activity of a single class of PSM. These tree species were also present in the home ranges of all females. We excluded *E. tereticornis* and *E. platyphylla* from our analyses, as they comprised a relatively small proportion of possums' diets and contained varying concentrations of FPCs, which may have confounded our results. Furthermore, *E. platyphylla* is deciduous, so it was not available as a food source year-round. Testing for differences between home ranges of individual females confirmed significant variation in the mean concentrations of our four leaf chemistry measures (for N digestibility,  $F_{14, 583} = 4.53$ ,  $P < 0.001$ ; for DM

digestibility,  $F_{14,583} = 9.62$ ,  $P < 0.001$ ; for PEG-BC,  $F_{14,583} = 4.15$ ,  $P < 0.001$ ; for total N,  $F_{14,583} = 4.67$ ,  $P < 0.001$ ). There was no relationship between possum home range size and any of these measures of nutritional quality.

Univariate models showed that the digestible N concentration of *E. drepanophylla* was significantly negatively correlated with PEG-BC ( $r^2 = 0.078$ ,  $F_{1,544} = 47.0$ ,  $P < 0.001$ ) and positively correlated with total leaf N ( $r^2 = 0.422$ ,  $F_{1,544} = 398.3$ ,  $P < 0.001$ ). A multivariate model containing both explanatory variables revealed that they both had a significant effect on digestible N concentration ( $r^2 = 0.441$ ,  $F_{2,543} = 216.28$ ,  $P < 0.001$ ). Total N explained a greater proportion of the variation in the model than did PEG-BC (coefficient of multiple determination =  $0.402 + 0.042 = 0.444$ ) (Sokal and Rohlf 1995). However, concentrations of digestible N were far more variable than were concentrations of total N (coefficient of variation for total N = 10.16%; digestible N = 30.75%) in *E. drepanophylla*, indicating that total N alone does not capture the full extent of nutritional variation that animals face. Our NIRS predictions for N digestibility in the presence and absence of PEG revealed that tannins reduced the N digestibility of *E. drepanophylla* foliage by 7–76% (mean = 36.25%).

#### Effects of plant chemistry on breeding success

Having demonstrated variation in the nutritional quality of individual home ranges, we tested whether these differences were reflected in the reproductive performance of females. Univariate logistic regression models showed that the likelihood of breeding increased with increasing mean digestible N concentrations (deviance = 17.82,  $P < 0.001$ ,  $n = 14$ ; Fig. 1a) and mean DM digestibility (deviance = 11.25,  $P < 0.001$ ,  $n = 14$ ; Fig. 1b) in the trees available to each female, but mean total N and mean PEG-BC had no effect. Females in better condition produced more offspring than did those in poorer condition (deviance = 5.86,  $P = 0.016$ ,  $n = 17$ ; Fig. 1c), but there was no effect of maternal age or size. In multivariate analyses, the best performing model was the one that included mean digestible N concentration and body condition (AIC = 13.2). However, the models including digestible N concentration and DM digestibility ( $\Delta$ AIC = 1.9) and a combination of digestible N, DM digestibility, and body condition ( $\Delta$ AIC = 1.5) also received substantial support. The model including digestible N alone was the only univariate model to receive substantial support ( $\Delta$ AIC = 0.1).

#### Effects of plant chemistry on offspring growth rates

We found a significant positive relationship between the mean digestible N concentration in trees available to each female and the growth rates of her offspring (Wald = 6.02,  $P = 0.014$ ,  $n = 27$ ; Fig. 2), although none of the other nutritional variables had an effect. We also found a positive relationship between mother's head length and

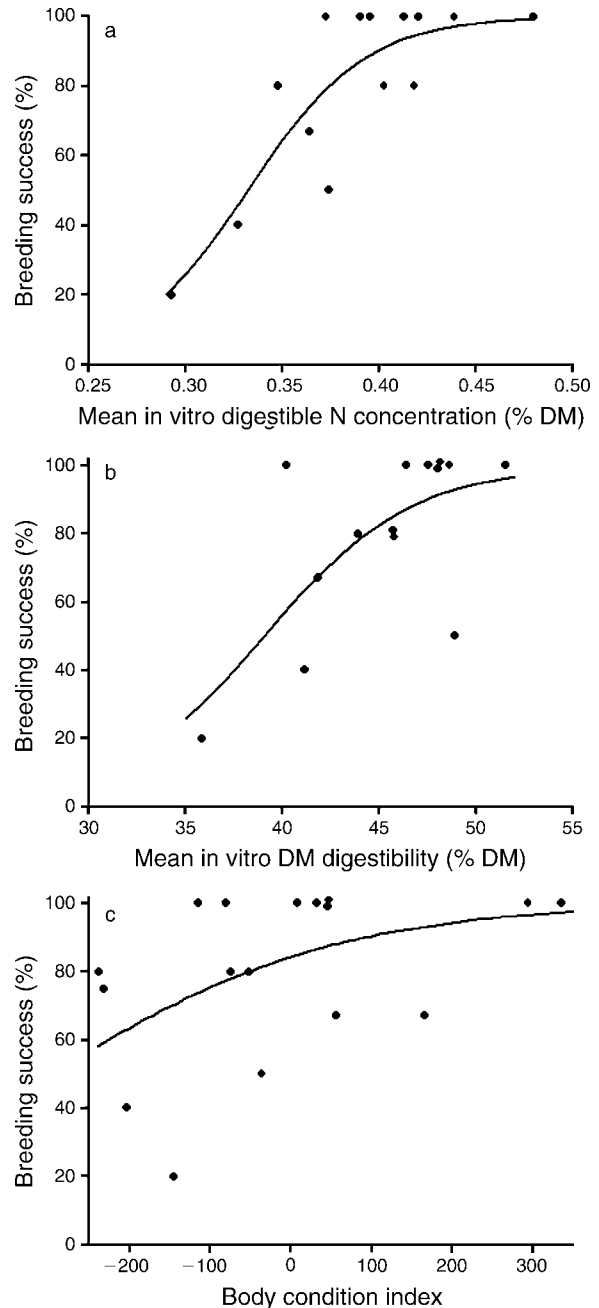


FIG. 1. Relationship between breeding success of female common brushtail possums (*Trichosurus vulpecula*) and (a) mean in vitro digestible nitrogen (N) concentrations in the trees available ( $P < 0.001$ ,  $n = 14$  breeding females), (b) mean in vitro dry matter (DM) digestibility of foliage in trees available ( $P < 0.001$ ,  $n = 14$  breeding females), and (c) body condition index ( $P = 0.016$ ,  $n = 17$  breeding females). The solid lines show the logistic regression models. Overlapping points have been offset for clarity. The body condition index is based on the residuals from a semiparametric local polynomial regression (LOESS regression) model of body mass on head length with the software package R (base package; R Core Development Team 2004).

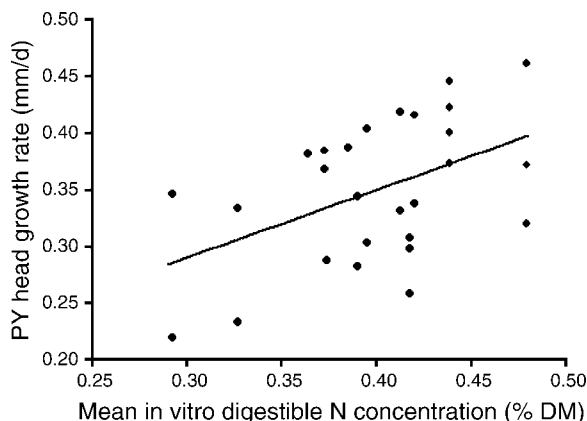


FIG. 2. Linear mixed model of the relationship between the head growth rates of pouch young (PY) of 13 female possums and mean in vitro digestible nitrogen (N) concentrations in each female's home range ( $P = 0.014$ ,  $n = 27$  pouch young).

PY growth (Wald = 5.84,  $P = 0.016$ ,  $n = 27$ ), but no effect of maternal age, body condition, or offspring sex. In a multivariate model, both digestible N concentration and maternal size were highly significant ( $P < 0.001$ ).

#### DISCUSSION

Our study describes a new approach to measuring the nutritional value of forests to herbivores and its implications for animal fitness. The central finding was that availability of in vitro digestible N, mediated by the interaction between tannin and N concentrations, is correlated with reproductive success in a population of folivores. We found that seemingly subtle differences in the availability of N between the home ranges of individual possums predicted very large differences in reproductive success. Our results indicate that female possums with home ranges containing trees with greater N availability invest more in reproduction, most likely as a result of improved nutritional status. Furthermore, those with access to higher concentrations of digestible N are likely to provide more nutrition to their young, resulting in faster growth, which translates to increased offspring fitness. Our results suggest a link between the combined effects of plant nutrient concentration and chemical defense, and reproductive fitness, which is important for explaining patterns of distribution and abundance in a range of plant-mammal systems.

The effect of home range quality on offspring growth rates in our model was very large, amounting to about a 40% increase in head growth over the measured range of digestible N concentrations. Early growth of mammals is implicated in traits such as size at maturity and future breeding potential (Kerr et al. 2007), so these differences could be expected to create wide variation in phenotypes and fitness among adults, even for animals coexisting in the same habitat, under the same environmental conditions. In many mammal species, faster-growing offspring are weaned earlier than others in their cohort,

conferring a lifelong advantage to these individuals (Smith et al. 1997, Festa-Bianchet et al. 2000, Kerr et al. 2007). Not surprisingly, we also found that larger mothers produced faster-growing young, which suggests that the benefits that females derive from better quality ranges are passed on to their young. Because female possums inherit home ranges from their mothers (Johnson et al. 2001), our results suggest that these effects may be persistent across generations. However, longer-term studies are necessary to reveal the precise mechanisms by which nutritionally mediated differences in offspring fitness impact on population dynamics over many years.

The effect of maternal range quality on fecundity in our model was greater still, with a fivefold difference observed. Our models revealed that although DM digestibility was important, digestible N concentration was the most powerful predictor of breeding success. Body condition was also weakly, but significantly, correlated with breeding success, although we found no relationship with other life history traits, such as age or size, which further emphasizes the importance of maternal nutrition. The result is a large skew in reproductive output, which at the scale of foraging ranges creates a mosaic of highly productive patches close to unproductive ones. This suggests that some home ranges are effectively demographic sources, where surplus individuals can be produced. In contrast, although there is sufficient food for possums to exist in other areas of the site, it appears that they provide suboptimal conditions for reproduction, which may have important consequences for population dynamics at a larger scale.

Ecologists recognize that foliar nutrition is likely to be involved in regulating the distribution and abundance of folivores, but studies that convincingly demonstrate an effect of specific nutritional parameters are exceedingly rare. Many have emphasized the importance of N to animal populations (White 1993, Pettorelli et al. 2001), but we found no effect of total N on animal performance, despite the fact that significant variation in N concentrations was apparent between individual possum home ranges. Similarly, there was no relationship between reproductive success and tannin concentrations (PEG-BC) per se. Rather, our approach integrating the effects of tannins on N availability was the key to unraveling the relationship between nutrition and reproduction. We found that the digestible N concentrations in the dominant tree species were negatively correlated with PEG-BC and positively correlated with total leaf N. Although total N was a stronger predictor of digestible N than was PEG-BC, this variable alone could not explain the observed variation in reproductive success. This suggests that both tannins and N do contribute to food quality, but it is only by considering them in combination that their effects on animal performance are revealed. Furthermore, we demonstrated that tannins were responsible for

substantially reducing N availability in the dominant tree species (up to 76%), which suggests a role for tannins in determining overall nutritional quality. The advantage of our approach is that by quantifying the concentration of N that is actually available for the animal to use, we can consider the nutritional quality of foliage from the perspective of the animal and interpret our results in a more ecologically meaningful way.

Using this approach, we were able to demonstrate a strong correlation between the nutritional value of plants and the demographic response of wild herbivores, which until now has remained elusive. Studies of folivorous primates have shown that protein: fiber ratios in leaves are positively correlated with animal abundance at a number of scales (Chapman et al. 2002, 2004, Wasserman and Chapman 2003). However, the problems of measuring fiber in tannin-rich plants and the potential biases introduced by ratios suggest that caution should be used with this approach. Furthermore, these studies have focused on population density, without consideration of how aspects of plant chemistry impact on the causal mechanisms underlying population dynamics, i.e., the reproductive rate. Many factors, including foliar nutrition, may interact to determine herbivore abundance (Oates et al. 1990), but we have shown how plant chemistry affects fecundity, which is a key determinant of population density. By considering variation in food quality at an intermediate spatial scale, we were able to measure the relevant demographic parameters to reveal such effects. At a larger scale these effects may not be clearly apparent, as they may be obscured by predation and other density-dependent factors.

Although our study was essentially correlative, the strength of our results inspires confidence that the digestible N concentrations of the tree species considered are important determinants of reproductive success in this possum population. The plant community structure across the site was homogenous, with the six species studied dominating the foraging environment of possums, and consequently comprising the majority of their diets. We consider it unlikely that the other tree species at the site would be critical to determining reproductive success, as they were low in abundance and constituted a relatively small proportion of possums' diets. Similarly, we are convinced that nutritional parameters are primarily responsible for the patterns observed, because factors such as predation risk and availability of shelter sites were uniform across the site. The response in maternal condition and pouch-young growth rates that we observed suggests that it is the transfer of nutrients from mother to offspring that determines variation in reproductive success in this species. There remains the possibility that fecundity and offspring growth are simply the result of increased female fitness, which may be genetically predetermined. However, the subtle variation in home range quality that we observed was a far more powerful predictor of reproductive success than

any of the life history traits measured, including size and condition. It is also unlikely that fitter females select the best quality home ranges and therefore gain the nutritional benefits, as home ranges are maternally inherited (Johnson et al. 2001).

Previous studies of captive and domesticated animals have found that manipulating the nutritional quality of foods significantly influences their reproductive success (e.g., Batzli 1986, Min et al. 1999). Similarly, food supplementation experiments have demonstrated effects of maternal nutrient intake on life history traits and demography in mammal species, such as squirrels (Boutin 1990, Kerr et al. 2007). However, we have demonstrated a relationship consistent with a strong role for maternal nutrition in a wild population, where animals must make complex foraging decisions among a large number of trees that are available to them. These findings provide a strong basis for future studies, not only of marsupials and eucalypts, but in a wide range of plant-mammal systems, including primates. Our approach to quantifying nutritional quality is also appealing as it is applicable in ecosystems where other PSMs prevail. This is because rather than simply measuring tannin concentrations, our method incorporates the effects of other factors that may limit N digestibility, such as cell wall constituents or other digestibility-reducing PSMs. Our approach may explain why mammal populations persist in some areas and not others.

Furthermore, foliar tannin concentrations increase and N concentrations decrease under conditions of elevated CO<sub>2</sub> in many plants (Lawler et al. 1997, Kanowski 2001, Mattson et al. 2004), resulting in large changes in digestible N. This may have strong detrimental effects on populations of folivorous marsupials in the wet tropics bioregion of Australia as atmospheric CO<sub>2</sub> concentrations rise (Williams et al. 2003). Therefore, our demonstration of how interactions between tannins and N influence the population processes of folivores may enable us to predict future patterns of herbivore distribution.

The variation in reproductive success that we observed is highly correlated with the availability of nutritional components in plants. There is ample evidence from this and other systems that levels of PSMs with a defensive function vary widely both within and between plant species, but the implications for herbivore populations are not understood. Our results show that the scale of this variation in eucalypts creates ecologically significant differences between feeding ranges of animals, which could impose strong effects on variation in individual reproduction and survival and ultimately determine distribution and abundance of herbivores at a landscape scale.

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## LITERATURE CITED

- Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pages 267–281 in B. N. Petrov and F. Csaki, editors. Second International Symposium on Information Theory. Akademiai Kiado, Budapest, Hungary.
- Andrew, R. L., R. Peakall, I. R. Wallis, and W. J. Foley. 2007. Spatial distribution of defense chemicals and markers and the maintenance of chemical variation. *Ecology* 88:716–728.
- Batzli, G. O. 1986. Nutritional ecology of the California vole: effects of food quality on reproduction. *Ecology* 67:406–412.
- Bignell, C. M., P. J. Dunlop, and J. J. Brophy. 1997a. Volatile leaf oils of some Queensland northern Australian species of the genus *Eucalyptus*. (Series II). Part II. Subgenera (a) *blakella*, (b) *corymbia*, (c) unnamed, (d) *idiogenes*, (e) *monocalyptus* and (f) *symphyomyrtus*. *Flavour and Fragrance Journal* 12:277–284.
- Bignell, C. M., P. J. Dunlop, J. J. Brophy, and J. F. Jackson. 1997b. Volatile leaf oils of some Queensland and northern Australian species of the genus *Eucalyptus*. (Series II). Part I. Subgenus *Symphyomyrtus*, Section *Adnataria*: (a) Series *Oliganthe*, (b) Series *Ochrophloiae*, (c) Series *Moluccanae*, (d) Series *Polyanthemae*, (e) Series *Paniculatae*, (f) Series *Melliodorae* and (g) Series *Porantheroideae*. *Flavour and Fragrance Journal* 12:19–27.
- Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems and the future. *Canadian Journal of Zoology* 68:203–220.
- Brenes-Aguedas, T., and P. D. Coley. 2005. Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species. *Oikos* 108:410–420.
- Bryant, J. P. 2003. Winter browsing on Alaska feltleaf willow twigs improves leaf nutritional value for snowshoe hares in summer. *Oikos* 102:25–32.
- Chapman, C. A., L. J. Chapman, K. A. Bjorndal, and D. A. Onderdonk. 2002. Application of protein-to-fiber ratios to predict colobine abundance on different spatial scales. *International Journal of Primatology* 23:283–310.
- Chapman, C. A., L. J. Chapman, L. Naughton-Treves, M. J. Lawes, and L. R. McDowell. 2004. Predicting folivorous primate abundance: validation of a nutritional model. *American Journal of Primatology* 62:55–69.
- Cork, S. J. 1992. Polyphenols and the distribution of arboreal, folivorous marsupials in *Eucalyptus* forests of Australia. Pages 653–663 in R. W. Hemingway and P. E. Laks, editors. *Plant polyphenols: synthesis, properties, significance*. Plenum Press, New York, New York, USA.
- Cork, S. J., I. D. Hume, and T. J. Dawson. 1983. Digestion and metabolism of a natural foliar diet (*Eucalyptus punctata*) by an arboreal marsupial, the koala (*Phascolarctos cinereus*). *Journal of Comparative Physiology B, Biochemical, Systemic, and Environmental Physiology* 153:181–190.
- DeGabriel, J. L., I. R. Wallis, B. D. Moore, and W. J. Foley. 2008. A simple, integrative assay to quantify nutritional quality of browses for herbivores. *Oecologia* 156:107–116.
- Festa-Bianchet, M., J. T. Jorgenson, and D. Reale. 2000. Early development, adult mass, and reproductive success in highborn sheep. *Behavioral Ecology* 11:633–639.
- Foley, W. J., A. McIlwee, I. Lawler, L. Aragones, A. P. Woolnough, and N. Berding. 1998. Ecological applications of near infrared reflectance spectroscopy a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance (review). *Oecologia* 116:293–305.
- Fox, L. R., and B. J. Macauley. 1977. Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia* 29:145–162.
- Ganzhorn, J. U. 1992. Leaf chemistry and the biomass of folivorous primates in tropical forests. Test of a hypothesis. *Oecologia* 91:540–547.
- Hooge, P. N., and B. Eichenlaub. 1997. Animal movement extension to ArcView. Version 1.1. Alaska Biological Science Center, U.S. Geological Survey, Anchorage, Alaska, USA.
- Iason, G. 2005. The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. *Proceedings of the Nutrition Society* 64:123–131.
- Isaac, J. L. 2005. Life history and demographics of an island possum. *Australian Journal of Zoology* 53:195–203.
- Isaac, J. L., and C. N. Johnson. 2003. Sexual dimorphism and synchrony of breeding: variation in polygyny potential among populations in the common brushtail possum, *Trichosurus vulpecula*. *Behavioral Ecology* 14:818–822.
- Johnson, C. N., M. Clinchy, A. C. Taylor, C. J. Krebs, P. J. Jarman, A. Payne, and E. G. Ritchie. 2001. Adjustment of offspring sex ratios in relation to the availability of resources for philopatric offspring in the common brushtail possum. *Proceedings of the Royal Society of London B* 268:2001–2005.
- Kanowski, J. 2001. Effects of elevated CO<sub>2</sub> on the foliar chemistry of seedlings of two rainforest trees from north-east Australia: implications for folivorous marsupials. *Austral Ecology* 26:165–172.
- Kerle, J. A. 2001. Possums. The brushtails, ringtails and greater glider. University of New South Wales Press, Sydney, Australia.
- Kerr, T. D., S. Boutin, J. M. LaMontagne, A. G. McAdam, and M. M. Humphries. 2007. Persistent maternal effects on juvenile survival in North American red squirrels. *Biology Letters* 3:289–291.
- Lawler, I. R., W. J. Foley, and B. M. Eschler. 2000. Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* 81:1327–1338.
- Lawler, I. R., W. J. Foley, I. E. Woodrow, and S. J. Cork. 1997. The effects of elevated CO<sub>2</sub> atmospheres on the nutritional quality of *Eucalyptus* foliage and its interaction with soil nutrient and light availability. *Oecologia* 109:59–68.
- Lawler, I. R., J. Stapley, W. J. Foley, and B. M. Eschler. 1999. Ecological example of conditioned flavor aversion in plant–herbivore interactions: Effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *Journal of Chemical Ecology* 25:401–415.
- Marsh, K. J., W. J. Foley, A. Cowling, and I. R. Wallis. 2003. Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*). *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 173:69–78.
- Mattson, W. J., K. Kuokkanen, P. Niemela, R. Julkunen-Tiitto, S. Kellomaki, and J. Tahvanainen. 2004. Elevated CO<sub>2</sub> alters birch resistance to *Lagomorpha* herbivores. *Global Change Biology* 10:1402–1413.
- McKey, D., P. G. Waterman, C. N. Mbi, J. S. Gartlan, and T. T. Struhsaker. 1978. Phenolic content of vegetation in two African rain forests: ecological implications. *Science* 202:61–63.
- McLean, S., and W. J. Foley. 1997. Metabolism of *Eucalyptus* terpenes by herbivorous marsupials. *Drug Metabolism Reviews* 29:213–218.



- Min, B. R., W. C. McNabb, T. N. Barry, P. D. Kemp, G. C. Waghorn, and M. F. McDonald. 1999. The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in sheep during late summer and autumn. *Journal of Agricultural Science* 132:323–334.
- Moore, B. D., and W. J. Foley. 2005. Tree use by koalas in a chemically complex landscape. *Nature* 435:488–490.
- Moore, B. D., W. J. Foley, I. R. Wallis, A. Cowling, and K. A. Handasyde. 2005. A simple understanding of complex chemistry explains feeding preferences of koalas. *Biology Letters* 1:64–67.
- Moore, B. D., I. R. Wallis, J. Wood, and W. J. Foley. 2004. Foliar nutrition, site quality and temperature affect foliar chemistry of tallowwood (*Eucalyptus microcorys*). *Ecological Monographs* 74:553–568.
- Oates, J. F., G. H. Whitesides, A. G. Davies, P. G. Waterman, S. M. Green, G. L. Dasilva, and S. Mole. 1990. Determinants of variation in tropical forest primate biomass: new evidence from West Africa. *Ecology* 71:328–343.
- Pettorelli, N., J. M. Gaillard, P. Duncan, J. P. Ouellet, and G. Van Laere. 2001. Population density and small-scale variation in habitat quality affect phenotypic quality in roe deer. *Oecologia* 128:400–405.
- Robbins, C. T., T. A. Hanley, A. E. Hagerman, O. Hjeljord, D. L. Baker, C. C. Schwartz, and W. W. Mautz. 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology* 68:98–107.
- Scrivener, N. J., C. N. Johnson, I. R. Wallis, M. Takasaki, W. J. Foley, and A. K. Krockenberger. 2004. Which trees do wild common brushtail possums (*Trichosurus vulpecula*) prefer? Problems and solutions in scaling laboratory findings to diet selection in the field. *Evolutionary Ecology Research* 6:77–87.
- Smith, B. L., R. L. Robbins, and S. H. Anderson. 1997. Early development of supplementally fed, free-ranging elk. *Journal of Wildlife Management* 61:26–38.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry. The principles and practice of statistics in biological research*, Third edition. W. H. Freeman, New York, New York, USA.
- Sorensen, J. S., J. D. McLister, and M. D. Dearing. 2005. Novel plant secondary metabolites impact dietary specialists more than generalists (*Neotoma* spp.). *Ecology* 86:140–154.
- Villalba, J. J., F. D. Provenza, and J. P. Bryant. 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? *Oikos* 97:282–292.
- VSN International. 2006. *GenStat. Ninth edition*. VSN International, Hemel Hempstead, UK.
- Wallis, I. R., and W. J. Foley. 2005. The rapid determination of sideroxydonals in *Eucalyptus* foliage by extraction with sonication followed by HPLC. *Phytochemical Analysis* 16: 49–54.
- Wallis, I. R., M. L. Watson, and W. J. Foley. 2002. Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Australian Journal of Zoology* 50:507–519.
- Wasserman, M. D., and C. A. Chapman. 2003. Determinants of colobine monkey abundance: the importance of food energy, protein and fibre content. *Journal of Animal Ecology* 72:650–659.
- White, T. C. R. 1993. *The inadequate environment: nitrogen and the abundance of animals*. Springer-Verlag, Berlin, Germany.
- Williams, S. E., E. E. Bolitho, and S. Fox. 2003. Climate change in Australian tropical rainforests: an impending environmental catastrophe. *Proceedings of the Royal Society B* 270: 1887–1892.
- Winter, J. W. 1980. Tooth wear as an age index in a population of the brush-tailed possum *Trichosurus vulpecula* (Kerr). *Australian Wildlife Research* 7:359–363.

#### APPENDIX

A table showing near-infrared reflectance spectroscopy (NIRS) modified partial least squares regression models for predicting foliar concentrations of polyethylene glycol (PEG)-binding component (PEG-BC), nitrogen (N), dry matter digestibility (DMD), N digestibility, and digestible N (*Ecological Archives* E090-050-A1).